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•论著•

人光老化皮肤中circRNA的表达谱及功能分析

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[摘要]目的 探究环状RNA (circRNA) 在人光老化皮肤中的表达谱及功能。方法 收集面部光老化皮肤 (实验组) 与臀部/腹部皮肤 (对照组), 通过circRNA芯片分析, 比较人光老化组与非光老化组皮肤circRNA的表达谱, qPCR验证芯片测序结果。结果 鉴定出202个差异circRNA。GO/KEGG分析显示差异circRNA母本基因富集于细胞外基质、胶原代谢等通路有关。qPCR证实hsa_circRNA_102207在实验组显著下调 ($P \leq 0.001$)。结论 光老化皮肤中异常表达的circRNA可能通过调控细胞外基质重塑、胶原组织及光老化关键通路参与病理过程。

[关键词] 人光老化皮肤; 环状RNA; 胶原蛋白; 弹性蛋白; circRNA芯片分析

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Expression Profile and Functional Analysis of circRNA in Photoaged Human Skin

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[Abstract]**Objective** To investigate the expression profile and functional roles of circular RNA (circRNA) in photoaged human skin. **Methods** Facial photoaged skin tissues (experimental group) and hip/abdomen skin tissues (control group) were collected. circRNA microarray analysis was performed to compare circRNA expression profiles between photoaged and non-photoaged skin groups, and qPCR was used to verify the microarray results. **Results** A total of 202 differentially expressed circRNAs were identified. GO/KEGG pathway analysis showed that the parental genes of differentially expressed circRNAs were enriched in pathways related to extracellular matrix and collagen metabolism. qPCR confirmed that hsa_circRNA_102207 was significantly downregulated in the experimental group ($P \leq 0.001$). **Conclusion** Abnormally expressed circRNAs in photoaged skin may participate in the pathological process by regulating extracellular matrix remodeling, collagen organization, and key photoaging pathways.

[Key words] Photoaged human skin; Circular RNA; Collagen; Elastin; circRNA microarray analysis

皮肤光老化 (photoaging of skin) 是长期紫外线 (UVA/UVB) 暴露导致的真皮层损伤, 临床表现为皱纹、弹性下降及恶性肿瘤风险增加^[1-3]。其核心机制涉及胶原降解和弹性纤维结构紊乱, 其中UVA通过诱导氧化应激和MMPs激活加速ECM破

坏^[4-6]。尽管针对胶原代谢的调控分子已有研究, 但ECM动态平衡的多层次调控网络仍不明确。近年来, circRNA因其共价闭合环状结构和高稳定性, 在疾病调控中备受关注。circRNA通过竞争性结合miRNA调控靶基因表达, 在疾病标志物和治

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疗靶点开发中优势显著^[7-9]。研究显示, circRNA在肿瘤和心血管疾病中具有调控功能^[10, 11], 在皮肤领域则与黑色素瘤发生相关^[12], 但其在光老化中的作用尚待阐明。本研究课题组前期研究发现, circRNA-859267通过吸附miR-29c拮抗胶原降解^[13], 芯片筛选发现慢性光损伤模型中128个差异circRNA^[14]。而circCOL-ELNs可协同抑制胶原减少和弹性蛋白沉积^[15]。然而现有研究局限于体外和动物模型, 人光老化皮肤circRNA表达谱仍属空白。本研究采用circRNA芯片技术筛选人光老化皮肤差异表达circRNA, 通过qRT-PCR验证测序结果可靠性, 旨在建立circRNA特征谱, 为光老化机制探讨和circRNA靶向治疗提供理论依据。

1 材料与方法

1.1 样本采集与分组 本研究于2021年1月-2022年12月在中山大学附属第七医院开展, 实验标本选自皮肤外科手术中废弃的皮肤组织。实验组为4例面部光老化皮肤(年龄55~75岁), 经Visa皮肤检测仪评估具有显著皱纹、红血丝及色素沉着; 对照组为4例非曝光部位(臀部/腹部)皮肤, 无光损伤表现。排除标准包括合并代谢性疾病(血糖异

常)、免疫性疾病(红斑狼疮)或皮肤肿瘤史, 以及近6个月使用免疫抑制剂或光敏药物。

1.2 总RNA提取、质控与完整性检测 总RNA通过Trizol法提取, 氯仿分层后异丙醇沉淀RNA。使用Nanodrop ND-1000检测RNA纯度(OD 260/280>1.8), 1%琼脂糖凝胶电泳验证完整性(28S/18S条带清晰)。

1.3 circRNA芯片分析 为富集circRNA, 采用Rnase R消化线性RNA(37 °C 15 min), 随后使用Arraystar Human circRNA V2.0芯片(8×15K)进行杂交, Agilent G2505C扫描仪获取信号, GenePix Pro 6.0软件提取原始数据。R语言limma包进行背景校正和分位数归一化, 筛选差异circRNA(FC≥1.5, $P<0.05$)。通过Miranda和TargetScan数据库预测靶基因, GO和KEGG分析通路富集。

1.4 qRT-PCR验证 候选circRNA通过qRT-PCR验证, 使用PrimeScript RT试剂盒合成cDNA, 针对hsa_circRNA_102207设计发散引物(GAPDH为内参), SYBR Green法扩增。反应条件为95 °C预变性5 min; 每个循环包括95 °C持续10 s、60 °C持续30 s, 共进行40个循环; 熔解曲线分析确认特异性。采用 $2^{-\Delta\Delta C_t}$ 法计算相对表达量。qPCR引物序列见表1。

表1 qPCR 引物序列

Gene	Forward primer sequence 5' → 3'	Reverse primer sequence 5' → 3'
GAPDH	AGAAGGCTGGGGCTCATTTG	GCAGGAGGCATTGCTGATGAT
hsa_circRNA_102207	GATACTGGCAGAGCGGAAGC	ACGACTCGTCGGGGAAGTAA

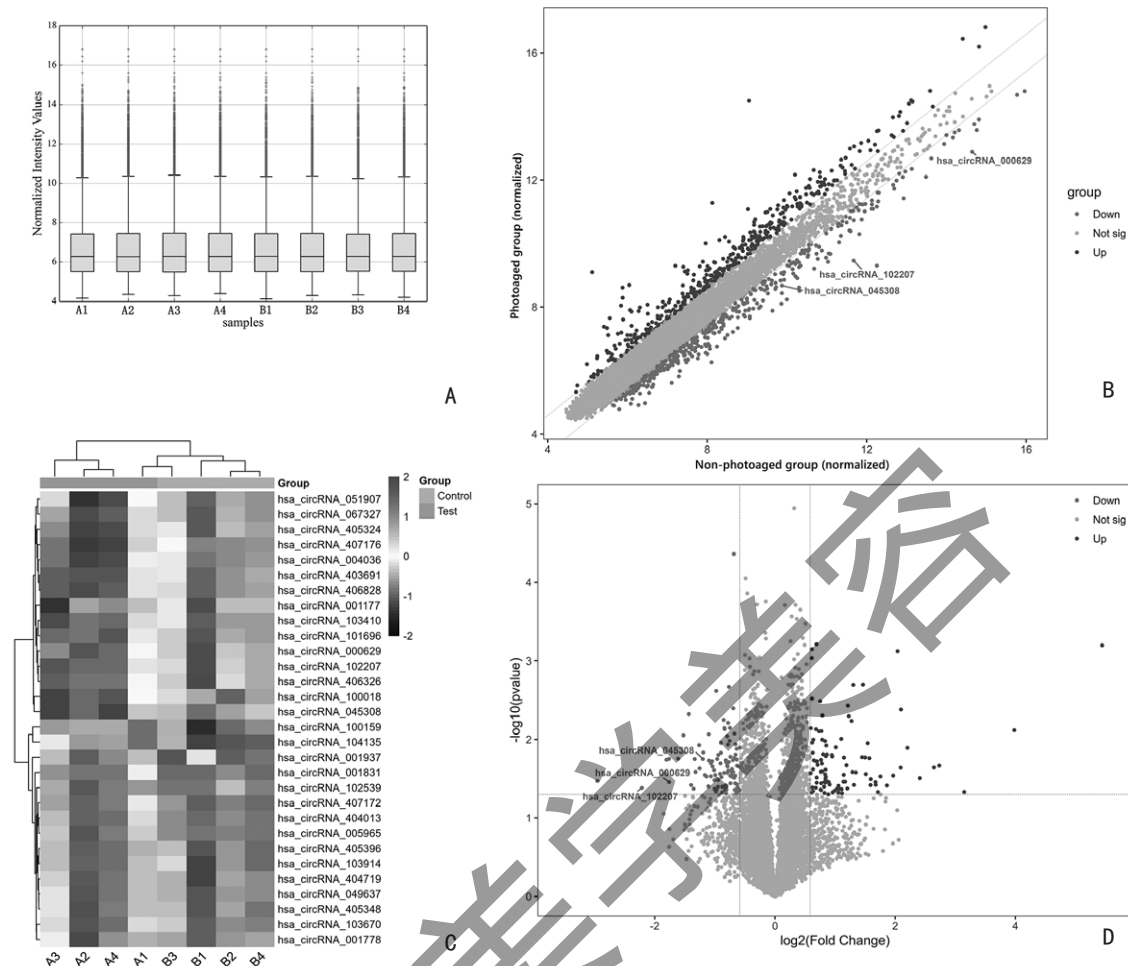
1.5 统计学方法 circRNA芯片表达数据采用R(4.1.1版)软件分析, GraphPad Prism 9.0进行t检验分析。以倍数变化≥1.5且 $P<0.05$ 作为统计学差异判定标准。

2 结果

2.1 差异circRNA表达谱分析 芯片分析显示, 与非光老化组相比, 光老化组存在202个差异circRNA(100个上调, 102个下调)。其中hsa_circRNA_102207、hsa_circRNA_045308和hsa_circRNA_000629差异最显著(见图1)。表2为前20个差异最显著的circRNAs, 其中10个呈上调表达, 10个呈下调表达。

2.2 GO和KEGG通路分析 GO分析显示, 差异circRNA母本基因显著富集于ECM相关通路: 生物过程包括胶原纤维组织、ECM组装; 细胞组分涉及胶原三聚体复合物和弹性纤维; 分子功能集中于ECM结构成分。KEGG通路分析显示, PI3K-AKT、ECM-受体互作及AGE-RAGE信号通路为关键调控网络(见图2)。

2.3 qRT-PCR验证 在差异circRNA中, 选取差异最显著的hsa_circRNA_102207进行后续qRT-PCR验证, 结果显示与阴性对照组相比, hsa_circRNA_102207在人体皮肤光老化组中的表达水平显著下调($P\leq 0.001$), 与芯片结果一致(见图3)。



注：A：人光老化皮肤组和非光老化组中 circRNAs 表达谱的分布没有差异；B：散点图显示了组间 circRNA 表达的变化；C：层次聚类分析表明两组样本可明显区分；D：火山图显示多数差异 circRNA 分布于高置信区间。

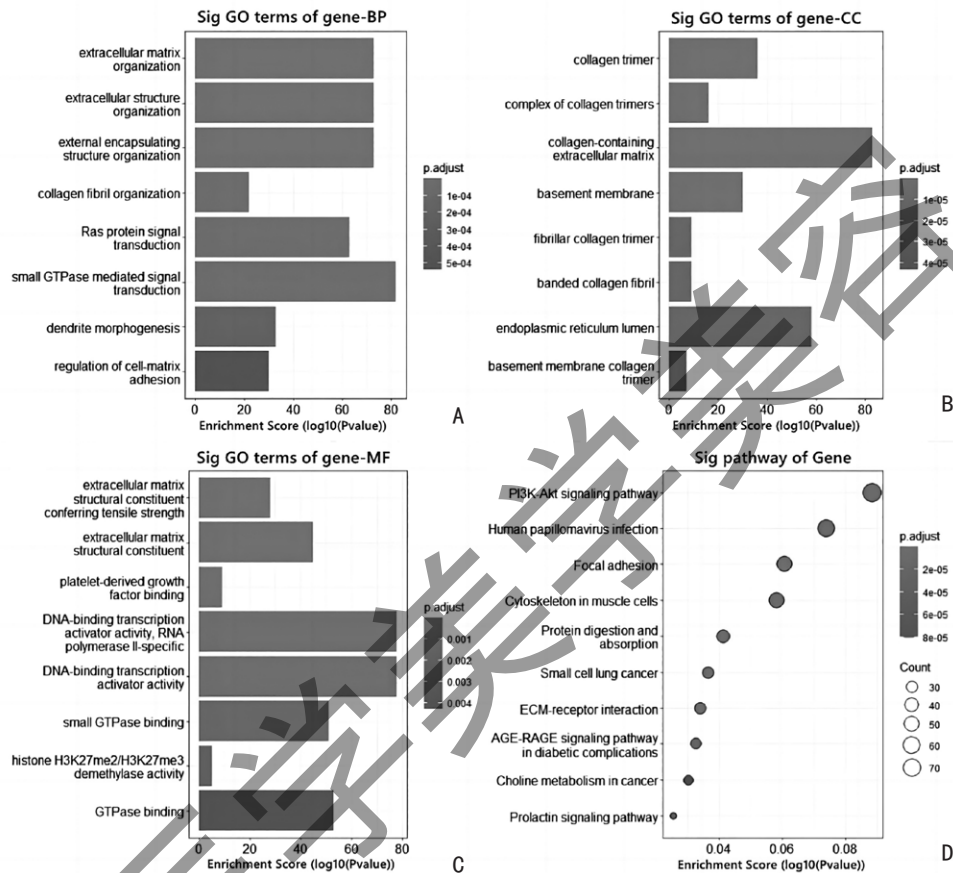
图1 人光老化皮肤组织中 circRNA 表达谱

表2 组间差异表达的前20个异常 circRNA

circRNA	Regulation	FC (abs)	GeneSymbol	P-value
hsa_circRNA_001830	up-regulated	43.506 262 6	SCRIB	0.000 632 748
hsa_circRNA_404012	up-regulated	15.799 733 9	BRF2	0.007 620 241
hsa_circRNA_103669	up-regulated	8.866 522 6	CNOT6L	0.046 619 178
hsa_circRNA_405395	up-regulated	6.650 592 5	SCAPER	0.021 560 299
hsa_circRNA_103913	up-regulated	6.256 179 8	MCTP1	0.022 774 003
hsa_circRNA_049636	up-regulated	5.313 248 3	CALR	0.031 072 248
hsa_circRNA_005964	up-regulated	4.607 434	PAPSS1	0.012 820 998
hsa_circRNA_407171	up-regulated	4.275 745 7	RP11-182N22.7	0.004 163 354
hsa_circRNA_404718	up-regulated	4.262 508 3	MCM10	0.022 888 697
hsa_circRNA_100158	up-regulated	4.111 970 4	CSMD2	0.000 632 748
hsa_circRNA_405324	down-regulated	7.755 574 3	STARD9	0.033 800 742
hsa_circRNA_102207	down-regulated	4.642 446 6	AFMID	0.041 465 148
hsa_circRNA_403691	down-regulated	3.495 226 8	LOC101927768	0.018 103 888
hsa_circRNA_407176	down-regulated	3.395 516 6	ZCCHC7	0.017 668 011
hsa_circRNA_000629	down-regulated	3.389 699 3	KIF18B	0.034 923 306
hsa_circRNA_406828	down-regulated	3.052 293 2	CDC40	0.017 564 17

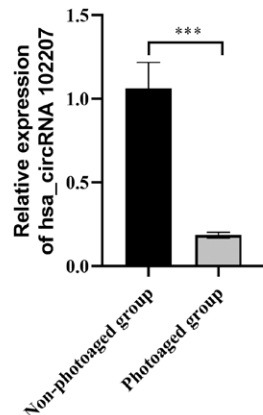
表 2 (续)

circRNA	Regulation	FC (abs)	GeneSymbol	P-value
hsa_circRNA_103410	down-regulated	2.873 420 2	LRIG1	0.008 863 804
hsa_circRNA_100018	down-regulated	2.700 791	GNB1	0.004 755 298
hsa_circRNA_051907	down-regulated	2.628 229 6	RPS11	0.038 181 704
hsa_circRNA_406326	down-regulated	2.554 655 3	QTRTD1	0.042 439 989



注: A: GOBP 显示 circRNA 的亲本基因转录 mRNA 基因富集分析; B: GOCC 显示细胞成分中 mRNA 基因富集分析; C: GOMF 显示了细胞功能中 mRNA 的基因富集分析; D: KEGG 通路分析揭示了可能参与皮肤光老化的通路。

图 2 GO 与 KEGG 通路富集分析



注: *** $P \leq 0.001$ 。

图 3 qPCR 检测组间 hsa_circRNA_102207 的表达情况 ($n=3$)

3 讨论

光老化作为紫外线 (UV) 长期暴露引发的皮肤病理进程, 主要由 UVA 和 UVB 通过诱导基质降解与氧化应激共同驱动。其不仅是外源性皮肤老化的核心诱因, 与皮肤松弛、皱纹形成等表型改变直接相关, 更被证实可促进日光性角化病、皮肤黑色素瘤等恶性疾病的发生发展^[3-5]。尽管胶原减少与弹性纤维紊乱已被明确为光老化的关键病理特征, 但针对二者协同调控的分子机制仍存在认知空白, 这限制了多靶点治疗策略的开发。

近年研究发现, circRNA 凭借其稳定的闭合

环状结构及miRNA海绵功能，在疾病调控中展现出独特潜力。前期研究通过人类真皮成纤维细胞（HDFs）及裸鼠慢性光损伤模型，首次鉴定出128个差异表达circRNA，并证实circCOL-ELNs可通过吸附miR-6732-5p等分子，双向调控I型胶原合成与弹性蛋白代谢。然而，上述结论均基于体外或动物实验，人光老化皮肤进程中circRNA的表达谱及其功能尚待解析。

本研究通过circRNA芯片技术系统分析人光老化皮肤组织，共鉴定202个差异表达circRNA（ $FC > 1.5$, $P < 0.05$ ），包含100个上调和102个下调circRNAs。生物信息学分析表明，差异circRNA的母本基因显著富集于细胞外基质（ECM）组装、胶原代谢等生物学过程，且与PI3K-AKT信号通路、ECM-受体相互作用等光老化相关通路密切相关。通过进一步qRT-PCR验证显示，hsa_circRNA_102207在光老化组中表达显著下调。以上结果提示hsa_circRNA_102207表达水平降低与人体皮肤光老化密切相关。结合既往发现的circRNA/miRNA/胶原调控轴，本研究提示hsa_circRNA_102207可能通过吸附特定miRNA调控胶原蛋白，从而参与光老化进程。

综上所述，本研究首次在人光老化皮肤中构建circRNA特征谱，突破了既往研究对体外模型的依赖。尽管样本量有限，但差异circRNA与动物模型数据的部分重叠，证实了跨物种调控机制的保守性。后续研究可通过扩大样本量、结合功能实验深入解析关键circRNA（如hsa_circRNA_102207）的作用机制，为开发靶向胶原蛋白重构的多通路干预策略提供理论依据。

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